PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 12(2): 1-4
https://doi.org/10.14719/pst.5024

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RESEARCH ARTICLE

LC/MS analysis and cytotoxicity activity of oyster on different cancer cell line

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OPEN ACCESS

ARTICLE HISTORY
Received: 11 September 2024
Accepted: 03 January 2025
Available online
Version 1.0 : 07 April 2025
Version 2.0 : 15 April 2025

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
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CITE THIS ARTICLE
Rana Hussein K, Rasha E, Wrood SA. LC/MS analysis and cytotoxicity activity of oyster on different cancer cell line. Plant Science Today. 2025; 12(2): 1-4. https://doi.org/10.14719/pst.5024

Abstract

Continuous attempts and studies have been conducted to discover a new agent that is highly effective against cancer cell with fewer side effects. One of these important new sources is marine organisms. A promising marine resource, reported in Chinese pharmacopeia as having antitumor properties, is the oyster shell. This research was designed to evaluate the cytotoxicity effect of oyster shell extract against three different cancer cells, first a sterile, 0.22 µM syringe filter was used to filter 1000 mg of oyster shell dissolved in dimethyl sulfoxide. The stock extract was stored at -80°C and then the active ingredients were identified using liquid chromatography-mass spectroscopy (LC-MS), while the anti-proliferative activity of oyster shell extract was evaluated by 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide assay. The analysis of oyster shell extract by LC-MS confirmed the presence of many active compounds such as coumarin, unsaturated fatty acids and glycosides. The marine oyster demonstrated significant cytotoxic activity against prostate cancer PC3 cells, with an IC_{50} value of 284 µg/mL. It exhibited modest cytotoxic activity against lung cancer cells (A549) and Abelson murine leukemia cells in mice, respectively. The detected cytotoxicity of oyster extract against various cancer cell lines may open the door for future research on cytotoxic agents for cancer control.

Keywords

cytotoxicity activity; liquid chromatography-mass spectroscopy; oysters

Introduction

Cancer is highly invasive and fatal disease, with the rate of morbidity increasing every year worldwide (1). Various treatment methods, such as chemotherapy, radiotherapy, immunotherapy and surgery have been employed to prevent, control the spread of and even cure cancer (2). Most of these strategies have failed to target cancer cells, specifically leading to severe side effects such as cardiotoxicity, neurotoxicity, gastrointestinal toxicity (GIT), renal toxicity and others (3). Continuous efforts and studies have been conducted to discover a new agent that are highly effective against cancer cell with minimal side effect (4, 5). Natural products are crucial and valuable sources for anticancer agents, as they are highly effective through various mechanisms. Additionally, some agents have been synthetically modified to enhance their activity or reduce side effects (6). The unique and complex chemical structures of natural products enable some to act on various cancer cells, such as alkaloids, lignan, terpenoids and flavonoid are well-known for their potent anticancer properties by interfering with the metabolic pathways of cancer cells (7, 8). These

compounds are primarily isolated from plants, bacteria and fungi and ongoing research is continued to identify new resources (9). One of these significant new sources is marine organisms. Indeed, about seven approved drugs and many others are under clinical trial (10, 11). A promising marine resource is reported in the Chinese pharmacopeia for its antitumor properties is the oyster shell (12).

Oysters possess a high nutritional and medicinal value due to their rich content, including many amino acids (like taurine), proteins, vitamins (A, C, D, E, B6 and B12), minerals (such as zinc, selenium, iron, potassium, magnesium and calcium), omega3 and polysaccharides (13, 14). Numerous nutraceutical benefits of oyster have been reported, such as improving immunity, rejuvenation, cardioprotective, aphrodisiac, weight loss and improving enzymes activity (15). Also, the calcium content of oyster shell makes them an excellent supplement for osteoporosis and calcium deficiency (16).

Materials and Methods

Preparation of oyster shell extract

A sterile 0.22 µM syringe filter was used to filter 1000 mg of oyster shell dissolved in dimethyl sulfoxide. The stock extract was stored at -80°C (17).

Instrumentation and MS parameters

Active compounds present in the oyster shell were identified using a Bruker Daltonik UPLC conjugated with ESI-Q-TOF (Bremen, Germany), along with high resolution Bruker TOF MS for m/z identification of each analyte after chromatographic separation and the conditions used are reported in Table 1.

Table 1. The conditions of LC/MS instrument

Voltage capillary	2500 V
Nebulizer gas	2.0 bar
Nitrogen flow rate	8 L/min
Dry temperature	200 °C
Mass accuracy	< 1 ppm
Mass resolution	50000 high resolution
TOF repetition rate	up to 20 kHz
UHPLC column	Bruker solo 2.0_C-18
Flow rate	51*10^-2 mL / min
Column temperature	40°C
Analyte	(A) water with 0.05% formic acid and (B) acetonitrile
Gradient	0-27 minutes: linear gradient from 5% to 80% B; 27–29 minutes: 95% B; 29.1 minutes: 5% B
Analysis duration	35 min on positive and 35 min on negative mode
Injection volume	3 µL
Sample preparation	Sample was diluted with 2.0 mL DMSO and completed to 50 mL by acetonitrile then centrifuged at 2000 rpm /1.0 min, 1.0 mL of sample are then put in sampler and 3.0 µL are injected.

Cell cultures

In this study two different cancer cell lines of human origin, in addition to mouse RAW 264.4 cells were examined: non-small cell lung cancer cells (A549) and prostate cancer cells (PC-3). These cell lines were procured from ATCC (Manassas, VA, USA). All were maintained in Dulbecco's Modified Eagle's Medium (DMEM) controlled by using a mixture of 1% antibiotics composed of penicillin + streptomycin and 10% fetal bovine serum (FBS). Finally, they were kept at room temperature in a 5% carbon dioxide incubator with approximately 96% humidity (18).

Cell proliferation assay

The MTT assay was conducted according to the manufacturer's instructions to assess the anti-proliferative activity of oyster extract. Approximately, about five thousand cells from each cell line were seeded into a 96-well plate, followed by the addition of 0.01 mL medium. The plate was incubated overnight in 5% carbon dioxide incubator. Next a mixture of 0.01 mL medium + oyster extract was added to each cell, with the extract applied in a series of final concentrations ranging from 0.5 -270 mg/mL. The culture was then re-incubated in a 5% carbon dioxide incubator for 48 hours. After incubation, the medium was removed from each well and 10 µL of MTT kit stock solution (5 mg/mL in PBS) was added. The culture was incubated for an additional four hours. Subsequently, the MTT solution and medium were removed and 100 µL of DMSO (dimethyl sulfoxide) was added to terminate the reaction. A multimode plate reader (Glomax-Promega, USA) was employed to measure the absorbance value of the cell suspension at 560 nm (19, 20).

Results and Discussion

Chromatography result

The analysis of oyster extract by LC-MS confirmed the presence of several active compounds, including coumarin, unsaturated fatty acids and glycosides as shown in the chromatogram (Fig. 1, Table 2).

Oyster shell extract inhibits cancer cells proliferation

Fig. 2 demonstrates that the presence of oyster shell extract caused a decrease in cell survival, with the extent of the decrease being directly proportional to the dosage. The marine oyster demonstrated a significant increase in cytotoxic activity against prostate cancer PC3 cells, with an IC_{50} value of 284 µg/mL. It exhibited modest cytotoxic action against lung cells (A549) and mice Abelson murine leukemia cells (RAW 264.7). The observed cytotoxicity of oyster extract is correlated to the synergistic activity between its active compounds (21). Multi-target cancer treatment is possible, as coumarin derivatives have been widely reported for their cytotoxicity and linoleic acid has demonstrated cytotoxic activity against various cancer cells (22-27). Also, the antioxidant and antitumor potential of kaempferol and quercetin glycosides have been reported in numerous studies (28-30).

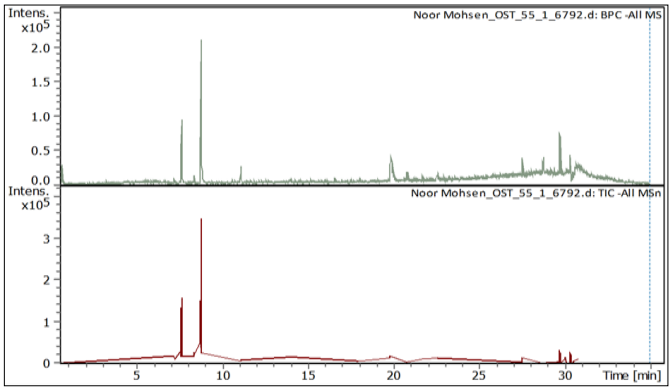

Fig. 1. The chromatogram of oyster.

Table 2. Active compounds present in oyster extract

Retention time [min]	Name of compound
6.97	(4 or 7) Hydroxy-coumarin plus hydrate
29.6	10E, 12Z-Linoleic acid
29.22	(Z)-3-Hydroxyoctadec-7-enoic acid (NMR)
7.07	3-O-Neohesperidoside kaempferol (NMR)
5.38	3-O-Neohesperidoside-7-rha kaempferol (NMR)
4.82	3-O-Neohesperidoside-7-rha quercetin (NMR)

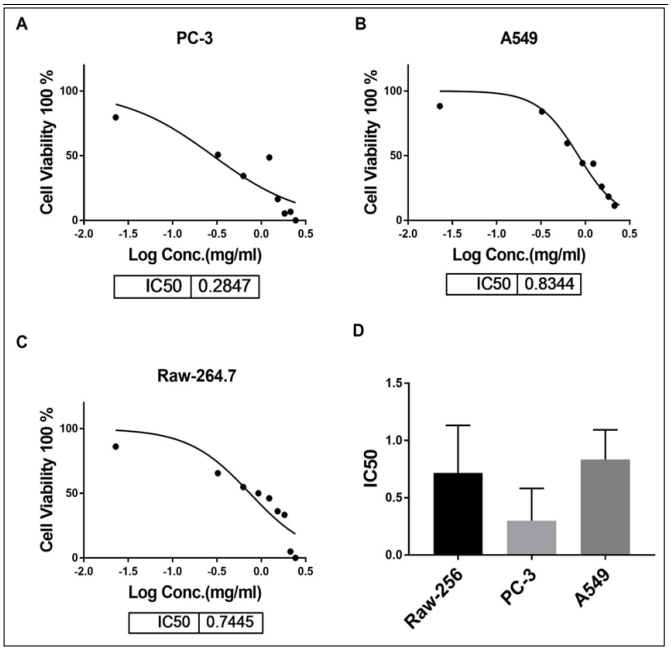

Fig. 2. Oyster's effect on cancer cell proliferation (A) PC-3, (B) A549 and (C) RAW-264.7, (D) IC_{50} of extract. Data are the mean ± SD of triplicate determinations.

Conclusion

Oyster shell shows significant cytotoxic activity against different cell lines and the detected cytotoxicity of oyster extract against various cancer cells may open the door for future analysis on cytotoxic agents for cancer control.

Authors' contributions

RE participated in the sequence alignment and drafted the manuscript. WSAK participated in the design of the study and performed the statistical analysis. RHK conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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